REMARKS/ARGUMENTS

Claims 99, 101, 104-113, 116, 120, and 142-144 are pending in the application. Claims 101 and 120 have been amended as further discussed below, and claims 143 and 144 have been amended for clarification. New claim 145 has been added. Applicants acknowledge with appreciation the Examiner's reconsidcration and withdrawal of one of the previously made rejections (Office Action, page 2, #3). However, Applicants note that the teachings regarding GM-CSF come from the inventors' own work as described in the application and not from the Inaba *et al.* reference, as has been discussed previously in detail (see, *e.g.*, the Amendment filed August 14, 2003 and the Amendment filed June 2, 2004). Further, Applicants note that working Examples 6 and 7 demonstrate the effects on human cells of culture in media containing GM-CSF as described (see pages 74-78 and 78-79 of the present Application No. 09/073,596).

Support for the claim amendments is discussed in more detail below. No new matter has been added by way of amendment. Reconsideration and reexamination of the claims are respectfully requested.

The Invention

The claimed invention is described throughout the application and relates to improved compositions and methods for providing them which involve culturing dendritic cell precursors *in vitro* in the presence of GM-CSF, which ultimately produces mature dendritic cells. Optionally, the methods and compositions can further involve exposing the cells to antigen, which produces antigen-activated mature dendritic cells which express modified antigens. Both these cells and the modified antigens they produce are embodiments of the invention. One of skill in the art will appreciate from the description of the invention in each application in the priority chain that the invention has a number of aspects and embodiments, including those presently claimed. For example, these and other aspects of the invention are described in the first-filed priority application (App. No. 07/861,612); see the "Summary of the Invention" on pages 7 through 10 and elsewhere, including the Abstract of the Disclosure, which states in part:

> "A method for producing proliferating cultures of dendritic cell precursors is provided. Also provided is a method for producing mature dendritic cells in culture from the proliferating dendritic cell

> precursors. The cultures of mature dendritic cells provide an effective means of producing novel T cell dependent antigens comprised of dendritic cell modified antigens or **dendritic cells pulsed with antigen which antigen is processed and expressed on the antigen-activated dendritic cell.** The novel antigens of the invention may be used as immunogens for vaccines or for the treatment of disease."

In view of this and additional support in the first-filed priority application (App. No. 07/861,612) and continuing in applications in the priority chain up through the present application (App. No. 09/073,596), Applicants respectfully submit that support is provided for the various aspects and embodiments of the invention listed above and those presently claimed.

The Claim of Priority Should Be Accepted; and

The Claims Meet the Requirements of § 112, First and Second Paragraphs

The Office Action maintains that the benefit of priority to earlier applications should be denied because "neither the '612 nor the '357 applications disclose the cells being cultured with an antigen as is recited in the last step of Claims 101 and 120" (Office Action at page 2, #4, first paragraph, referring to the priority Applications Nos. 07/861,612 and 07/981,357). The Office Action also rejects claims 99, 101, 104-113, 116, 120, and 142-144 as failing to satisfy the written description requirement (Office Action at page 6, #11) in view of the last portion of claim 101. Applicants respectfully disagree with the analysis and conclusion set forth in the Office Action because the '612 priority application does support the claims and further because statements in the outstanding Office Action appear to be at odds with the last Response filed by Applicants on March 10, 2009.

For example, the Office Action states that "Applicant now cites page 3, lines 8-14 of the '612 specification..." to support the "wherein" clause of pending claim 101 (see Office Action at page 2, last two paragraphs) and concludes that "[t]his step has not been found in either of the '612 nor '357 applications." However, the last Response filed by Applicants on March 10, 2009 does not mention page 3, lines 8-14 of the '612 specification. Rather, in the last Response (page 6, first full paragraph), Applicants noted support for the "wherein" clause of claim 101 in the '612 priority application at page 22, lines 10-20, which essentially recite this clause as set forth, as follows:

> The antigen-activated dendritic cells of the invention are produced by exposing antigen, *in vitro*, to the dendritic cells prepared according to the method of the invention. Dendritic cells are plated in culture dishes and exposed to antigen in a sufficient amount and **for a sufficient period of time to allow the antigen to bind to the dendritic cells.**"

(see the '612 priority application on page 22, lines 10-20 (also found in the present specification on page 34, line 33 through page 35, line 3)). Claim 101 has been amended to recite this limitation as an active method step in order to address the rejection of the claim for indefiniteness (Office Action on page 6, #13). As amended, the last clause of claim 101 now requires "culturing the dendritic cells *in vitro* in the presence of an antigen for a time sufficient to allow the antigen to bind to the dendritic cells." Applicants maintain that the '612 passage quoted above provides the necessary support to satisfy the written description requirement and respectfully request that the rejection of the priority claim on this basis be reconsidered and withdrawn.

Further, in the previous response (page 6, last three paragraphs), Applicants also cited support for the second part of the last clause of pending claim 101, which was (and remains) "wherein the dendritic cells process the antigen to produce a modified antigen which is expressed by the dendritic cells." The portion of the '612 priority application cited in support of this limitation included original claims 36 and 17 of the '612 priority application, which were as follows:

> 36. A composition comprising antigen-activated dendritic cells **wherein dendritic cells prepared according to claim 17 are pulsed with an antigen** and **wherein the dendritic cells process the antigen to produce a modified antigen which is expressed by the dendritic cells.**
>
> 17. A method of producing a population of mature dendritic cells from proliferating cell cultures comprising:
>
> a) providing a tissue source comprising dendritic cell precursors;
>
> b) treating the tissue source to obtain a population of cells suitable for culture *in vitro*;
>
> c) culturing the tissue source on a substrate in a culture medium comprising GM-CSF to obtain nonadherent cells and cell clusters;
>
> d) subculturing the nonadherent cells and cell clusters to produce cell aggregates comprising proliferating dendritic cell precursors;
>
> e) serially subculturing the cell aggregates one or more time to enrich the proportion of dendritic cell precursors; and

> f) continuing to culture the dendritic cell precursors for a period of time sufficient to allow them to mature into mature dendritic cells.

Applicants maintain that these original claims from the '612 priority application provide the necessary support for this limitation in pending claim 101. Further, while the cited support is located on different pages of the '612 priority application, a careful reading of the '612 application shows that the various passages all relate to the "antigen-activated dendritic cells" described throughout the application and presently claimed.

The Office Action also states (page 3) that "[n]either cite discloses culture in GM-CSF which Applicant argues later in the current remarks is the most important factor in generating the mature DCs of the instant claims." Discussion of support for this aspect of the invention was omitted in the previous response as it did not appear to be raised as an issue in the previous Office Action. However, as discussed extensively in previous responses, support for requiring culture in GM-CSF is found in all the applications in the priority chain, including the '612 priority application. As discussed in the Amendment filed August 5, 2008, page 10:

> "The importance of culture in GM-CSF is discussed throughout the '612 application, particularly, for example, on page 16, lines 2-6, which state that 'GM-CSF has surprisingly been found to promote the proliferation *in vitro* of precursor dendritic cells. Cells are cultured in the presence of GM-CSF at a concentration sufficient to promote the survival and proliferation of dendritic cell precursors.'"

The same language is found in the '357 specification at page 19, line 28 through page 20, line 3, and in the present application (App. No. 09/073,596) at page 25, lines 19 through 23. As discussed in various parts of the '612 application, including for example the Summary of the Invention, these methods produce mature dendritic cells *in vitro* in "amounts suitable for various immunological interventions for the prevention and treatment of disease" (see the '612 application at page 12, line 30 through page 13, line 3).

The Office Action reiterates that the benefit of Applicants' priority claim(s) of the present application are denied, reciting a block quote from a previous Office Action (see page 2, #4, first paragraph) which includes the statement that "the method step employed in instant Claim 101 comprising, 'treating the tissue source comprising dendritic cell precursors to increase the

proportion of dendritic cell precursors' is not found in the '612 application." Applicants respectfully note that support for this method step has been previously addressed and acknowledged. Particularly, the Amendment filed on August 5, 2008 at page 5, last paragraph, cited support for this step in the '612 priority application at page 13, lines 16-18 and page 13, lines 4-8; the subsequently issued Final Rejection of November 18, 2008 (at page 2, #3, second paragraph), stated that

> "In view of Applicant's citing of page 13 [of the '612 priority application] to support 'treating the tissue source comprising dendritic cell precursors to increase the proportion of dendritic cell precursors,' that ground for denying priority has been withdrawn."

Accordingly, Applicants respectfully request that the denial of the priority claim on this basis be reconsidered and withdrawn.

Support for the further limitations of claim 101 as currently amended can be found in the '612 priority application on page 7, line 22 through page 8, line 6, which are as follows:

> "This invention also claims a method of producing *in vitro* mature dendritic cells from proliferating cell cultures. The method comprises
> (a) providing a tissue source comprising dendritic cell precursor cells;
> (b) treating the tissue source to obtain a population of cells suitable for culture in vitro;
> (c) culturing the tissue source on a substrate in a culture medium comprising GM-CSF, or a biologically active derivative of GM-CSF, to obtain non-adherent cells and cell clusters;
> (d) subculturing the nonadherent cells and cell clusters to produce cell aggregates comprising proliferating dendritic cell precursors;
> (e) serially subculturing the cell aggregates one or more times to enrich the proportion of dendritic cell precursors; and
> (f) continuing to culture the dendritic cell precursors for a period of time sufficient to allow them to mature into mature dendritic cells."

(indentation of clauses (a) through (f) added for clarification)

A comparison of the above clauses (a) through (f) to amended claim 101 will show that this language appears in the amended claim. Amended claim 101 also includes additional

limitations, support for which is discussed above. Support for the additional limitations found in the dependent claims were discussed extensively in the Amendment filed August 5, 2008 (pages 10-13) and were not objected to in the present Office Action, so that discussion is not repeated here. In view of the above discussion of support for the various limitations and the above discussion regarding the various aspects of the invention (and also, for example, in the '612 application "Summary of the Invention" on pages 7 through 10), Applicants respectfully submit that the claims are fully disclosed in and supported by the priority '612 application (as well as in the other applications in the priority chain) and therefore meet the written description requirement. Accordingly, the benefit of priority of the '612 application should be accorded to the present claims.

Claim 120 was amended to address the rejection for indefiniteness (see Office Action at page 6, #13) and for clarification of the claimed invention. Support for claim 120 as amended can be found in the '612 priority application on page 12, lines 28-30; page 16, lines 3-6; page 18, lines 26-29; and page 22, lines 12-15. These passages are as follows in the '612 application: page 12, lines 28-30 state that "[t]his invention relates to a method of producing cultures of proliferating dendritic cell precursors which mature *in vitro* to mature dendritic cells"; page 16, lines 3-6 state that "[c]ells are cultured in the presence of GM-CSF at a concentration sufficient to promote the survival and proliferation of dendritic cell precursors"; page 18, lines 26-29 state that "[t]o further expand the population of dendritic cells, cell aggregates may be serially subcultured multiple times at intervals which provide for the continued proliferation of dendritic cell precursors"; and page 22, lines 12-15 state that "[d]endritic cells are plated in culture dishes and exposed to antigen in a sufficient amount and for a sufficient period of time to allow the antigen to bind to the dendritic cells." In view of this support in the '612 priority application, Applicants respectfully submit that claim 120 meets the requirements of patentability and is in condition for allowance.

In a further effort to advance prosecution and to provide claim language that is deemed to meet the written description and other requirements, Applicants have also added new claim 145. Support for method steps (a) through (g) is as discussed above for claim 101, although step (b) of claim 101 is not found in claim 145. Claim 145 finds further support in '612 original claims 17

and 36 (reproduced above) and in the '612 priority application on page 8, lines 15-19, which are as follows:

> "Another embodiment of the invention are **antigen-activated dendritic cells prepared according to the method of the invention which antigen-activated dendritic cells have been exposed to antigen and express modified antigens** for presentation to and activation of T cells."

The support for the claims in the '612 application discussed above is also found in every application in the priority chain, including the instant application (No. 09/073,596). In view of the support in the applications for the amended claims, Applicants respectfully submit that the claims are fully supported by the specification(s), including the first-filed priority application. Accordingly, it is respectfully submitted that the claims meet the written description requirement and that the priority claim should be given full weight.

The Rejections of Claims under 35 U.S.C. § 102 Should Be Withdrawn

The Office Action (page 3, #6) has maintained the rejection of claims 99, 101, 104-113, 116, 120, and 142-144 under 35 U.S.C. § 102(a) over Pancholi *et al.* (1992) *Immunology* 76: 217-224. While the Pancholi reference was published after the filing date of the '612 priority application and thus is not available as art against the present claims, the Office Action maintains (page 4, paragraph 7) the denial of the benefit of the priority claim. Applicants respectfully disagree with this conclusion and traverse the rejection.

As discussed above, independent claims 101 and 120 (and therefore also claims 99, 103-113, 116, and 142-144, all of which depend from or incorporate the limitations of claims 101 or 120) have been amended, and new claim 145 has been added, to more closely reflect the language of the '612 priority application. In view of the support in the '612 application for these claims, as discussed in detail above, Applicants respectfully submit that the claims are entitled to the '612 priority date (*i.e.*, April 1, 1992) and that Pancholi therefore is not available as prior art against the claims. Accordingly, Applicants request that this rejection of claims be reconsidered and withdrawn.

The Rejections of Claims under 35 U.S.C. § 103 Should Be Withdrawn

The Office Action (page 4, #9) has rejected claims 99, 101, 104-113, 116, 120, and 142-144 under 35 U.S.C. §103(a) over Inaba *et al.* (1990) in view of Steinman *et al.* ((1988) *Ann. N.Y. Acad. Sci.* 546: 80-90) and Markowicz and Engleman ((1990) *J. Clin. Invest.* 85: 955-961). The Office Action concludes (page 5, last paragraph) that

> "It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to add GM-CSF to a cell culture of DCs such as the mouse cultures of Inaba et al. and Steinman et al. or the human cultures of Steinman et al. and Markowicz and Engleman. The ordinarily skilled artisan would have added GM-CSF to DC cultures given the teachings of Steinman et al., that, DC 'maturation is driven by factors such as IL-1 and GM-CSF,' etc. and Markowicz and Engleman, that, 'GM-CSF ... profoundly affects the morphology and viability of DCs isolated from peripheral blood....' Accordingly, the GM-CSF-cultured DCs as claimed are obvious in view of the combined prior art."

Applicants respectfully disagree with this conclusion and traverse the rejection. The cited references do not render the claimed invention obvious because none of the references, nor any combination thereof, teaches the claimed compositions or the method steps specified in the claims that are necessary to produce them. The Inaba reference includes experiments on dendritic cells but does not teach anything about GM-CSF and also does not teach or suggest that dendritic cell precursors even exist. None of the references teach or suggest that culture of dendritic cell precursors *in vitro* in GM-CSF can be used to produce an enriched and expanded population of proliferating dendritic cell precursors which give rise to a population of mature dendritic cells.

The Office Action cites the Markowicz and Engleman reference as teaching that "'GM-CSF ... profoundly affects the **morphology and viability** of DCs isolated from peripheral blood...'" and concludes that one of skill would have been motivated to add GM-CSF to DC cultures (Office Action, page 5, last paragraph). However, rather than suggesting such a combination, the Markowicz and Engleman reference instead teaches away from the claimed invention and particularly teaches away from the use of GM-CSF to induce proliferation. Particularly, Markowicz and Engleman conclude that in the presence of GM-CSF, "the number

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of viable cells as well as the number of branched cells per well **remained stable over time, suggesting that GM-CSF does not cause DC to divide and proliferate**" (sentence bridging page 958-959; emphasis added). This is supported by Markowicz and Engleman's Figure 4 (on page 959), which shows that there was no significant increase in the number of viable cells (as well as differentiated DC) "throughout the culture period" (see Figure 4 legend on page 959). In view of this teaching by Markowicz and Engleman, one of skill in the art would not have added GM-CSF to Inaba's cell cultures to induce cell proliferation according to the method specified in the claims and so would not have been motivated to produce the claimed invention.

The third reference cited in the rejection of claims under §103 (Office Action (page 5, third paragraph) is the Steinman reference (Steinman *et al.* (1988) *Ann. N.Y. Acad. Sci.* 546: 80-90), which is cited in the Office Action as teaching:

> "the enrichment and culturing of both mouse and human immature DCs found in blood, as well as bone marrow (see pages 81-83) and that, 'maturation is driven by factors such as IL-1 and GM-CSF' (see page 83). The reference further teaches that 'GM-CSF is critical in mobilizing active DCs at the onset of a cell-mediated immune response' (see page 88)."

However, Applicants respectfully submit that the Steinman reference teaches away from the claimed invention because it states that GM-CSF has a role *in vivo* in **maturation** of cells in certain tissues and proposes that GM-CSF may be involved in **mobilizing** DCs at the onset of a cell-mediated immune response. The Steinman reference does not teach that GM-CSF is involved in stimulating cell proliferation and thus does not teach or suggest the method used to produce the claimed invention; accordingly, it does not teach or suggest the claimed cells.

Further, the Steinman reference states with regard to "immature forms of dendritic cells" (paragraph bridging pages 83 and 84) that:

> "The term 'immature' is used, because these populations must be cultured for 1-2 days before optimal levels of surface Ia and accessory function are expressed."

Thus, the Steinman reference teaches that "immature" DCs need only be cultured for 1-2 days, and that this time is necessary for maturation. The Steinman reference does not teach or suggest that culture of dendritic cell precursors in GM-CSF can produce a population of

proliferating dendritic cell precursors as taught by Applicants, or the mature dendritic cells resulting from such a process, as presently claimed.

Applicants respectfully note that MPEP §2113 requires that when product-by-process claims are examined, "[t]he structure implied by the process steps should be considered when assessing the patentability of product-by-process claims over the prior art, especially where the product can only be defined by the process steps by which the product is made, or where the manufacturing process steps would be expected to impart distinctive structural characteristics to the final product." Here, the claims are drawn to an *in vitro* composition of cells derived from an *in vitro* culture of an enriched and expanded population of proliferating precursors by a method comprising culture in GM-CSF, which surprisingly was found to promote that proliferation (see, *e.g.*, '612 application on page 16, lines 2-6, stating that "GM-CSF has surprisingly been found to promote the proliferation *in vitro* of precursor dendritic cells"; and on page 16, lines 23-24, stating that "[i]n the absence of GM-CSF, no colonies develop"). Thus, the '612 application (as well as the present specification) teaches that **culture of the cells *in vitro* in GM-CSF provides the enriched and expanded population of proliferating dendritic cell precursors required by the present claims, and these cells differ from previously reported cells**, for example, in their ability to take up antigen even after extended periods of culture.

As discussed in detail previously, the cells of the invention differ from previously reported cells. Specifically, for example, the fresh spleen cells taught by the cited Inaba reference differ in at least several significant ways from the *in vitro* compositions of the present invention (see Inaba *et al.* (1990) *J. Exp. Med.* 172: 631-640, as discussed previously, for example, in the previous Office Action of November 18, 2008 and the Office Action of 2 July 2002). First, as taught by the Inaba reference, fresh spleen cells can only take up antigens for a short time, and lose this ability in culture[1]. In contrast, the cells of the present invention can take up antigen after being cultured for many days (see, *e.g.*, Figure 13, showing uptake and expression of antigen after cells had been cultured for 6 days in GM-CSF). Further, because

[1] See Inaba *et al.* (1990), *e.g.*, at page 632, left column, first full paragraph: "As will be evident in Results, it was necessary to expose fresh rather than cultured dendritic cells to a foreign protein to successfully charge these APC with antigen." Also see page 632, right column, first paragraph of "Results" section: "[w]e conclude that freshly isolated dendritic cells can be successfully pulsed with a variety of soluble protein antigens in vitro, but that it is important to administer the antigen shortly after isolating the dendritic cells from the spleen."

fresh spleen cells like those of Inaba's lose their ability to take up antigen in culture, fresh spleen cells cultured without exogenous GM-CSF cannot give rise to enriched and expanded cell populations which take up antigen, as required by the present claims. The cells of the present invention further provide enriched and expanded cell populations in clinically useful quantities (see, *e.g.*, '612 application at page 7, first paragraph and the present specification at page 40, lines 25-28), a benefit resulting from Applicants' innovative step of culturing the cells in GM-CSF so as to obtain proliferating dendritic cell precursors. The methods taught by Inaba do not produce increased cell populations. Thus, the claimed invention has a number of advantages which are not taught or suggested in the prior art.

In summary, culture of the dendritic cell precursors in GM-CSF, as discovered by Applicants, is essential to the development of *in vitro* cultures of proliferating precursor cells and provides a number of advantageous properties to the resulting dendritic cells. None of the cited references has taught or suggested these critical features of the invention. Accordingly, the claimed invention cannot be rendered obvious by any of the cited references or any combination thereof and Applicants respectfully request that this rejection of the claims be withdrawn.

CONCLUSION

In view of the foregoing claim amendments and discussion, Applicants respectfully submit that the rejections of claims have been overcome and that the claims are in condition for allowance. However, if the Examiner believes that any further discussion of this communication would be helpful, he is encouraged to contact the undersigned by telephone.

Applicants note that in view of recent case law regarding citation of references, Applicants are also submitting on this date an Information Disclosure Statement for the instant application with a number of references that were cited in related cases.

No additional fees or extensions of time are believed to be due in connection with this communication except for those indicated in documents accompanying this paper. However, if any additional extensions of time are necessary for the consideration of this paper, such

extensions are petitioned under 37 CFR § 1.136(a). Please apply any charges that may be due for extensions of time or for net addition of claims to our Deposit Account No. 50-3187.

Respectfully submitted,

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CERTIFICATE OF FACSIMILE

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted on the date indicated below to the United States Patent and Trademark Office via facsimile transmission to (571)273-8300.

Signature

Dec. 8, 2009
Date